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In re Application of:)

Keith E. LeJeune et al.)

Serial No. 09/441,592)

Filed: November 17, 1999)

Art Unit: 1651)

Examiner: D. Naff)

Atty. Ref: 99-041)

ENZYME-CONTAINING
POLYURETHANES

Pittsburgh, Pennsylvania 15219

November 12, 2002

Box RCE
Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION OF KEITH E. LEJEUNE

I, Keith E. LeJeune, hereby declare and say as follows:

1. My academic experience, professional honors and publications are set forth in the *curriculum vitae* attached hereto as Exhibit A.

2. My work experience is set forth in the *curriculum vitae* attached hereto as Exhibit A.

3. I am one of the inventors of the subject matter of U.S. Patent Application Serial No. 09/441,592.

4. I am currently employed by Agentase, LLC, the assignee of the above-identified patent application.

5. I have reviewed Havens et al. cited by the Examiner in the Office Action dated October 7, 2002. In Havens et al., a very crude (impure) protein preparation was used at a maximum concentration of 5mg/g polymer. Although this is 0.5 wt% of protein preparation, the concentration of enzyme in that preparation is much lower. Although not indicated, the enzyme loading in the polymer of Havens et al. is quite low. Indeed, the low enzyme loading of Havens et al. can be demonstrated mathematically.

6. In that regard, the turnover number (or kcat) for the subject enzyme of Havens et al. (Parathion hydrolase from *P. diminuta*) on parathion is well established as 1,067 $\mu\text{mol}/\text{min}/\text{mg}$ enzyme. (See, for example, Dumas et al., 1989 J. Biol. Chem. 264, 19659-19665 and Dumas et al., 1990 Arch. of Biochem. Biophys., 277, 1, 155-159). The inventors of the present application have verified these findings in the laboratory.

7. In Havens et al, the polymer used to initiate the hydrolysis (shown in Figure 1) is described as a 5.5g polymer carrying 2.9 mg protein / g prepolymer, or approximately 16mg of protein. Havens et al. also describes that the standard assay was to apply substrate solutions to the polymers at 10-times the polymer mass, or in that case 55ml of parathion solution. Figure 1 shows a reduction of parathion concentration from 0.045 to 0.01 $\text{nmol}/\mu\text{L}$ in 5 minutes, or a rate of 0.007 $\text{nmol}/\mu\text{L}/\text{min}$ (or also 7 $\text{nmol}/\text{mL}/\text{min}$). Multiplying by the volume of 55 ml, one calculates the apparent reaction rate to be 385 nmol/min . Considering 16 mg of the protein prep was required to achieve this rate, the actual observed rate was 24 $\text{nmol}/\text{min}/\text{mg}$ protein.

8. If one conservatively assumes a low activity retention of enzyme of 1% during polymerization, the resulting rate in the Havens et al. experiment should have been 10.67 $\text{nmol}/\text{min}/\text{mg}$ enzyme, if the protein preparation of Havens et al. was pure enzyme. The division of their achieved rate (24 $\text{nmol}/\text{min}/\text{mg}$ protein) by that expected (10.67 $\text{nmol}/\text{min}/\text{mg}$ enzyme) for pure enzyme, provides the purity of the prep (0.024/10.67) or 0.002 mg enzyme/ mg protein.

This calculation indicates that the protocols described by Havens et al. employed a protein prep that was less than or equal to 0.2% pure enzyme. Enzyme loading would thus be less than or equal to 0.0058 mg enzyme/ g prepolymer ($[0.002 \text{ mg enzyme} / \text{mg protein}] * [2.9 \text{ mg protein} / \text{g prepolymer}]$) or .00058 wt percent enzyme loading ($0.0058 \text{ mg enzyme} / 1000 \text{mg prepolymer} * 100$). Even assuming an extremely low activity retention of 0.1%, the wt percent enzyme loading of the polymer of Havens et al. would be only .0058 wt%.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that willful false statements may jeopardize the validity of the referenced application or any patent issuing thereon.

Nov 12, 2002
Date

Keith E. LeJeune
Keith E. LeJeune



Keith E. LeJeune, Ph.D.

CEO, Agentase LLC

RECENT WORK HISTORY

Agentase, LLC – Pittsburgh, PA

Chief Executive Officer

Director of Research and Development, Co-founder

Advanced Extravascular Systems, Westwood, CA

Chief Scientist

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1998-1999

1998

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EDUCATION

B.S. Chemical Engineering (1994) University of Pittsburgh, Pittsburgh, PA.

M.S. Chemical Engineering (1996) University of Pittsburgh, Pittsburgh, PA.

Ph.D. Chemical Engineering, (1998) Carnegie Mellon University, Pittsburgh, PA.

Thesis Title: *Employing Enzymes in the Detoxification of Nerve Agent Chemical Weapons*

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SELECTED RECENT PUBLICATIONS AND RELEVANT PRESENTATIONS

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- LeJeune, K.E., Erbeltinger, M., Sensors for the Detection of an Analyte, US provisional patent application 60/328,524, Submitted Nov 2001.
- LeJeune, K.E. and Russell, A.J., US Patent No. 6,291,200, Enzyme-containing polymeric sensors. (September 18, 2001).
- Dravis, B.C., LeJeune, K.E., Hetro, A.D., Russell, A.J., *Enzymatic Dehalogenation of Gas Phase Substrates*, Biotechnol. Bioeng. 2000, 69(3), Pp. 235-241.
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- LeJeune, K.E., Wild, J.R., and Russell, A.J., *Nerve Agents degraded by enzymatic foams*, Nature, 1998, Vol. 396, no. 6697, Pp. 27-28.
- LeJeune, K.E., et al., *Increasing the Tolerance of Organophosphorus hydrolase to Bleach*, Biotech. Bioengr. 1999, Vol. 64, (2) Pp. 250-254
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- LeJeune, K.E., and Russell A.J., *Biocatalytic Nerve Agent Degradation, - A Battle for the 21st Century*, Biochemical Engineering X, Engineering Foundation Conference, Kananaskis, Alberta, Canada, May 1997.